

- 106 -

Claims

1. A purified MHC Class II polypeptide comprising covalently linked first and second domains, wherein:

5 the first domain is a human MHC class II β 1 domain and the second domain is a mammalian MHC class II α 1 domain and wherein the amino terminus of the second domain is covalently linked to the carboxy terminus of the first domain and wherein the MHC class II molecule does not include an α 2 or a β 2 domain; or

10 the first domain is a human MHC class I α 1 domain and the second domain is a mammalian MHC class I α 2 domain, and wherein the amino terminus of the second domain is covalently linked to the carboxy terminus of the first domain and wherein the MHC class I molecule does not include an α 3 domain.

15 2. The polypeptide of claim 1 wherein the covalent linkage between the first and second domains is provided by a peptide linker sequence.

20 3. The polypeptide of claim 1 wherein the polypeptide further comprises, covalently linked to the amino terminus of the first domain, a third domain comprising an antigenic determinant.

4. The polypeptide of claim 3, wherein the antigenic determinant is a peptide antigen.

25 5. The polypeptide of claim 4, wherein the covalent linkage between the first and third domains is provided by a peptide linker sequence.

6. The polypeptide of claim 1, further comprising an antigenic determinant associated with the polypeptide by non-covalent interaction.

- 107 -

7. The polypeptide of ~~claim 6~~, wherein the antigenic determinant is a peptide antigen.

8. The polypeptide of ~~claim 1~~ wherein the polypeptide further comprises a covalently linked detectable marker or toxic moiety.

9. The polypeptide of ~~claim 1~~, wherein the covalent linkage between the $\beta 1$ and $\alpha 1$ domains is provided by a peptide linker sequence.

10. A nucleic acid molecule encoding the polypeptide of ~~claim 1~~.

11. The nucleic acid of ~~claim 10~~, operably linked to a promoter.

12. A vector comprising the nucleic acid of ~~claim 10~~.

13. The vector of ~~claim 12~~, wherein the vector is a viral vector.

14. A host cell transformed with the nucleic acid of ~~claim 10~~.

15. A recombinant polypeptide comprising $\beta 1$ and $\alpha 1$ domains of a human MHC class II molecule wherein the amino terminus of the $\alpha 1$ domain is covalently linked to the carboxy terminus of the $\beta 1$ domain, and wherein the MHC class II molecule does not include an $\alpha 2$ domain or a $\beta 2$ domain.

16. The recombinant polypeptide according to ~~claim 15~~, wherein the polypeptide further comprises an antigenic determinant associated with the polypeptide by covalent or non-covalent interaction.

- 108 -

17. The recombinant polypeptide according to claim 16, wherein the antigenic determinant is covalently linked to the amino terminus of the β 1 domain.

18. The recombinant polypeptide according to claim 15, wherein the polypeptide further comprises a detectable marker or toxic moiety.

19. A recombinant polypeptide comprising a human MHC class I α 1 domain and a human MHC class I α 2 domain, and wherein the amino terminus of the α 2 domain is covalently linked to the carboxy terminus of the α 1 domain, and wherein the MHC class I molecule does not include an α 3 domain.

20. The recombinant polypeptide according to claim 19, wherein the polypeptide further comprises an antigenic determinant associated with the polypeptide by covalent or non-covalent interaction.

21. The recombinant polypeptide according to claim 20, wherein the antigenic determinant is covalently linked to the amino terminus of the α 1 domain.

22. The recombinant polypeptide according to claim 20, wherein the polypeptide further comprises a detectable marker or toxic moiety.

23. A pharmaceutical composition comprising a polypeptide according to claim 1, and a pharmaceutically acceptable carrier.

24. A recombinant polypeptide comprising only two domains of a human MHC class II peptide, wherein the two domains are an α 1 domain and a β 1 domain, wherein the amino terminus of the α 1 domain is covalently linked to the carboxy terminus of the β 1 domain.

25. The polypeptide of claim 24, wherein the covalent linkage between the $\alpha 1$ and $\beta 1$ domains is provided by a peptide linker sequence.

5 26. The purified MHC polypeptide of claim 24, wherein the MHC polypeptide is non-covalently associated with an antigen.

27. The purified MHC polypeptide of claim 24, wherein the MHC polypeptide is covalently associated with an antigen.

10

28. A recombinant polypeptide comprising only two domains of a human MHC class I peptide, wherein the two domains are an $\alpha 1$ domain and a $\alpha 2$ domain, wherein the amino terminus of the $\alpha 2$ domain is covalently linked to the carboxy terminus of the $\alpha 1$ domain.

15

29. The polypeptide of claim 28, wherein the covalent linkage between the $\alpha 1$ and $\alpha 2$ domains is provided by a peptide linker sequence.

20

30. The purified MHC polypeptide of claim 28, wherein the MHC polypeptide is non-covalently associated with an antigen.

31. The purified MHC polypeptide of claim 28, wherein the MHC polypeptide is covalently associated with an antigen.

25

32. A recombinant nucleic acid molecule, comprising first, second and third regions represented by the formula Pr-B-A, wherein:

Pr is a promoter sequence;

B is a coding sequence that encodes a $\beta 1$ domain of a human MHC class II molecule;

and

- 110 -

A is a coding sequence that encodes an $\alpha 1$ domain of a human MHC class II molecule;

wherein Pr is operably linked to B, and B and A comprise a single open reading frame.

5

33. A recombinant nucleic acid molecule, comprising first, second, third and fourth regions represented by the formula Pr-P-B-A, wherein:

Pr is a promoter sequence;

P is a coding sequence that encodes a peptide antigen;

10

B is a coding sequence that encodes a $\beta 1$ domain of a human MHC class II molecule; and

A is a coding sequence that encodes an $\alpha 1$ domain of a human MHC class II molecule;

wherein Pr is operably linked to P, and P, B and A comprise a single open reading frame.

15

34. A recombinant nucleic acid molecule, comprising first, second and third regions represented by the formula Pr-B-A, wherein:

Pr is a promoter sequence;

20

B is a coding sequence that encodes an $\alpha 1$ domain of a mammalian MHC class I molecule; and

A is a coding sequence that encodes an $\alpha 2$ domain of a mammalian MHC class I molecule;

25

wherein Pr is operably linked to B, and B and A comprise a single open reading frame, and wherein the open reading frame does not encode an $\alpha 3$ domain of a mammalian MHC class I molecule. /

35. A recombinant nucleic acid molecule, comprising first, second, third and fourth regions represented by the formula Pr-P-B-A, wherein:

- 111 -

Pr is a promoter sequence;

P is a coding sequence that encodes a peptide antigen;

B is a coding sequence that encodes an $\alpha 1$ domain of a human MHC class I molecule;

and

5 A is a coding sequence that encodes an $\alpha 2$ domain of a human MHC class I molecule;

wherein Pr is operably linked to P, and P, B and A comprise a single open reading frame, and wherein the open reading frame does not encode an $\alpha 3$ domain of a mammalian MHC class I molecule.

10 36. A method for detecting or quantifying in a biological sample the presence of T-cells having a receptor specific for a specified antigen, comprising:

contacting the biological sample with a recombinant polypeptide comprising either

(1) covalently linked $\beta 1$ and $\alpha 1$ domains of a human MHC class II molecule wherein the

15 carboxy terminus of the $\beta 1$ domain is covalently linked to the amino terminus of the $\alpha 1$ domain, and further comprising the specified antigen bound in a peptide binding groove formed by the $\beta 1$ and the $\alpha 1$ domain; or (2) a recombinant polypeptide comprising

covalently linked $\alpha 1$ and $\alpha 2$ domains of a human MHC class I molecule wherein the carboxy terminus of the $\alpha 1$ domain is covalently linked to the amino terminus of the $\alpha 2$ domain,

20 wherein the polypeptide does not include an $\alpha 3$ domain of a human MHC class I molecule and wherein the polypeptide further comprises the specified antigen bound in a peptide binding groove formed by the $\alpha 1$ and the $\alpha 2$ domain; and

detecting or quantifying the presence of specific binding of the recombinant polypeptide with said T-cells.

25

- 112 -

37. A method for reducing an immune response against an antigenic determinant in a subject, comprising:

administering a therapeutically effective amount of the polypeptide of claim 3, or of a nucleic acid encoding the polypeptide of claim 3; and subsequently presenting the antigenic determinant to the subject, wherein administration of the polypeptide or the nucleic acid sequence reduces the immune response when the antigenic determinant is presented in the subject.

10 38. The method of claim 37, wherein the reduced immune response is a decrease in an influx or proliferation of a T cell, a macrophage, a B cell, or an NK cell.

39. The method of claim 37, wherein the reduced immune response is a reduction in the expression of a cytokine.

15 40. The method of claim 37, wherein the reduced immune response is an induction of a T suppressor cell response.

20 41. A method for inducing an immunoregulatory cell against an antigenic determinant, comprising

administering a therapeutically effective amount of the polypeptide of claim 3 to the immunoregulatory cell; and subsequently presenting the antigenic determinant to the immunoregulatory cell;

25 wherein the presentation of the antigenic determinant results in an induction of the immunoregulatory cell.

- 113 -

42. The method of claim 41, wherein the immunoregulatory cell reduces inflammation and cellular recruitment when the antigen is subsequently encountered with an immunogenic stimulus.

5 43. The method of claim 41, wherein the antigenic determinant is a tissue specific antigenic determinant.

44. The method of claim 41, wherein the immunoregulatory cell is induced as compared to a control.

10

45. The method of claim 41, wherein the immunoregulatory cell is *in vivo*.

46. The method of claim 41, wherein the immunoregulatory cell is *in vitro*.

15

47. A method for inducing the expression of a cytokine in a mammalian T cell, comprising
contacting the T cell with an effective amount of the polypeptide of claim 3, thereby inducing the expression of the cytokine.

20

48. The method of claim 47, wherein the cytokine is IL-10.

49. The method of claim 47, wherein the cell is *in vivo*.

50. The method of claim 47, wherein the cell is *in vitro*.

25

- 114 -

51. A method of treating or preventing an immune-mediated disorder in a subject, comprising

administering to the subject a therapeutically effective amount of the polypeptide of claim 3 or of a nucleic acid encoding the polypeptide of claim 3;

wherein subsequent presentation of the antigenic determinant to an immune cell of the subject results in treatment or prevention of the immune-mediated disorder.

52. The method of claim 51, wherein the immune-mediated disorder is rheumatoid arthritis, chronic beryllium disease, insulin-dependent diabetes mellitus, throiditis, inflammatory bowel disease, uveitis, polyarteritis, Multiple Sclerosis or Myasthenia Gravis.

53. A pharmaceutical composition comprising the polypeptide of claim 3 in a pharmaceutically acceptable carrier.

54. A method of treating a disease caused by antigen-specific T-cells, comprising administering to a patient a composition comprising a polypeptide according to claim 3, or a nucleic acid encoding the polypeptide of claim 3, thereby treating the disease.

55. A method of activating a T cell in a subject, comprising administering a therapeutically effective amount of the polypeptide of claim 3, thereby activating the T cell.

56. The method of claim 55, wherein the subject is human.

57. The method of claim 55, wherein the T cell produces IL-10.

58. The method of claim 55, wherein the antigenic determinant is an antigenic determinant from a tumor cell.

Add
C3

Add
E5